CONTAINS TSCA CONFIDENTIAL BUSINESS INFORMATION

Microbial Commercial Activity Notice (MCAN) Submission

to the

U.S. Environmental Protection Agency
Office of Pollution Prevention and Toxics
Chemical Control Division
New Chemicals Notice Management Branch

TS Number: J-012-1WK8NE

Date of Submission: March 21, 2012

Submitter: [redacted]

Submitted to: TSCA Document Processing Center (7407)
Room L-100
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U.S. Environmental Protection Agency
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Certification of Information

I certify that to the best of my knowledge and belief: the company named in this submission intends to manufacture, import, or process for a commercial purpose, other than in small quantities solely for research and development, the microorganism identified in this submission. All information provided in the submission is complete and truthful as of the date of this submission. I am including with this submission all test data in my possession or control and a description of all other data known to or reasonably ascertainable by me as required by 40 C.F.R. § 725.160 or § 725.260.

[redacted]

[redacted]

[redacted]

Date

<u>CONTAINS TSCA CONFIDENTIAL BUSINESS INFORMATION</u>

<u>SUBSTANTIATION OF CONFIDENTIALITY FOR THE INFORMATION CLAIMED AS CONFIDENTIAL BUSINESS INFORMATION IN THE MCAN</u>

The following information is submitted in accordance with 40 C.F.R. § 725.94. A proposed generic name is provided for the microorganism identity in Section 1.4 of this MCAN pursuant to 40 C.F.R. §§ 725.80(a)(1) and 725.85(a)(3)(ii). A proposed category of use and a generic use description is provided in Section 1.5 of this MCAN pursuant to §§ 725.80(a)(2) and 725.88(b).

A. The nature of the Company's business is relatively unique in U.S. and international commerce. The technology is such that a competitor would be able to discern the production of the microorganism if Submitter Identity, Microorganism Identity, Process Information, Use, and Internal Company Documents (excluding health and safety studies) are publicly disclosed. A competitor, upon discovering this information, would be able to manufacture, sell or use the microorganism with no investment in research or development, all to our company's detriment. Consequently, such a disclosure is intolerable.

B. This information should be held in confidence indefinitely, until the technology is obsolete, or until the microorganism is widely known as a result of competing research.

C. Our company has kept guarded information related to Submitter Identity, Microorganism Identity, Process Information, Use, and Internal Company Documents (excluding health and safety studies) so that others cannot discover the commercial utility of this information. Only those with a need to know have access to this information.

D. Our company has disclosed information in these areas to outside legal counsel who appropriately protect its confidential status. Only those [redacted] on a need-to-know basis are aware of the association between the organism and its location of manufacture and production so that its commercial utility cannot be discovered. No confidential information or licenses under existing patents and patent applications have been disclosed or granted to others without secrecy agreements.

E. There are no advertising or promotional materials for the microorganisms. No Material Data Safety Sheet(s) is publicly available. No trade publications reference the microorganism. The Company's development of the microorganism has been held in strictest confidence such that no competitor is aware that this particular microorganism is in use. No Federal, local, or state agency or court has public files disclosing the Company's identity, process, or referenced internal documents in connection with the microorganism.

F. The Company, pursuant to 40 C.F.R. § 725.92(b) and § 725.95(e), claims as confidential, any reference to the microorganism's identity as well as any information that would facilitate the discovery of its identity in: (1) health and safety studies conducted by the submitter; and (2) published scientific journal articles submitted with the MCAN. Disclosure of the microorganism's identity would reveal confidential processing manufacturing trade secret, unrelated to health and the environment. Less specific identity information is sufficient to interpret the references provided, because the results and conclusions of the researcher are fully disclosed by the articles.

G. There are patents associated with the MCAN strain. However, the microorganism is only one of many microorganisms that have been disclosed in many of these patents. Thus, the identity of the microorganism, which is the subject of this MCAN, should be treated as confidential because these patents do not disclose the microorganism's identity, *per se.* Furthermore, the mere existence of such patents does not necessarily indicate that this microorganism or any other member of the category for which patent claims have been made, is utilized in U.S. commerce.

H. No Federal agency or court has ruled on the confidentiality of the microorganism.

I. A competitor, upon discovering this information, would be able to manufacture, sell or use the microorganism with no investment in research or development, all to our company's detriment. Consequently, such a disclosure is intolerable.

J. EPA disclosure of the information claimed as confidential would allow a competitor to enter the market more easily, benefitting greatly from our research and development expenditures. Competitors with the facilities, personnel and expertise to produce these microorganisms quickly, would benefit greatly at our expense, significantly reducing their own research and development time.

K. The microorganism will not leave the site of production in a viable state thereby allowing for testing in a form that is accessible to the public or its competitors. The cost to a competitor, in time and money, to develop appropriate use conditions would be approximately [redacted] The protection of the organism's confidentiality and secure handling will impede product analysis by others.

1.0 INTRODUCTION

1.1 Overview

This MCAN submission is for a specific genetically modified organism that will be used for [redacted] potential chemical uses.

The chemical substance produced [redacted]

The chemical substance that is the subject of this request, [redacted]

[redacted] Ongoing R&D efforts are conducted in compliance with the R&D exemption at 40 C.F.R §725.234 and 725.235 [redacted]

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[1] [redacted]

[redacted]

Figure 1a [redacted]

[redacted]

Figure 1b. [redacted]

1.2 Purpose of the MCAN

Pursuant to 40 C.F.R. § 725.105 [redacted] is filing this Microbial Commercial Activity Notice (MCAN) with the Environmental Protection Agency (EPA) for an [redacted]

[redacted] In addition, the modifications to the recipient strain meet the conditions for introduced genetic material at 40 C.F.R. § 725.421 for the Tier 1 exemption.

The production strain is currently intended for [redacted] which will meet the containment criteria to qualify for the full Tier 1 exemption. Future production may expand to include other facilities that will also meet the containment criteria to qualify for the full Tier 1 exemption. Given the low hazard potential of the production strain, we respectfully conclude in this submission that current operating conditions at large-scale, conventional fermentation processes will not present an unreasonable risk in association with the use of the notified strain.

1.3 Contact Information

In accordance with 40 C.F.R. § 725.155(c), the following information is provided.

Submitter: [redacted]
Address:

Contact: [redacted]

Technical Contact: [redacted]

An agent letter is provided as **Attachment 1**.

1.4 Proposed Generic Name

The explicit and formal biological name of the microorganism [redacted] A generic name for this microorganism that is in accord with 40 C.F.R. § 725.85 is **“microorganism modified**.**”** [redacted] The manufacturer of the MCAN microorganism considers as highly confidential the identity [redacted] This gene distinguishes the MCAN microorganism from more conventional [redacted] strains and contributes new and useful performance properties to the microorganism. Nondisclosure of the specific gene used to modify the microorganism is necessary to reduce the likelihood of a competitor manufacturing a similar product without the considerable investment in research and development required to develop such a product.

1.5 Proposed Use Category and Generic Use Description

[redacted] The submitter proposes the following generic use description: **“chemical production**.**”** This description protects from disclosure the confidential process and purpose of the manufacture while disclosing, with respect to exposure and release, the chemical to be produced.

2.0 MICROORGANISM IDENTITY INFORMATION

In accordance with 40 C.F.R. § 725.155(d), the following information is provided.

2.1 Recipient Strain Identification – [redacted]

The host organism, [redacted] Although [redacted] does not specify biosafety levels for their microorganisms, they identify strains that have the potential to be pathogenic. The parental strain [redacted] is not identified as being potentially pathogenic.

[redacted] was taken through clonal (single cell) purification and given the [redacted] Further information on the human health and environmental hazard assessment of [redacted] is presented in Sections 3.0 and 4.0 below.

[redacted] it is identified [redacted] and designated as Biosafety Level 1 (BSL-1). [redacted]

[redacted]

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[2] [redacted]

2.1.1 Morphological differences

Figure 2 demonstrates the morphological difference between [redacted]

[redacted]

Figure 2 [redacted]

2.1.2 Genetic divergence

[redacted] performed genotyping of [redacted] at the plastidic 23S rRNA locus. As seen below in Figure 3, the genome sequences of these two species are clearly different. The PCR method used to perform this genotyping can be used to distinguish [redacted] species.

[redacted]

Figure 3. [redacted]

Partial plastidic 23S rDNA sequences were generated for [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Figure 4 [redacted]

2.1.3 Biochemical/physiological differences

[redacted] grown under identical conditions were analyzed. The two strains exhibited very different growth rates, [redacted] These differences are illustrated in Figure 5 and Table 1.

[redacted]

[redacted]

Figure 5 [redacted]

Strain		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Day 1	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Day 3	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Day 5	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Table 1. [redacted]

2.1.2.1 Relatedness of [redacted]

In order to gain a more thorough understanding of the genetic relatedness of a variety of [redacted] we attempted to carry out genotyping of a highly divergent region of the plastidic 23S rRNA on 14 different isolates. Thirteen of these were from the [redacted] as shown in Table 2 below.

[redacted]	Species per source collection	Date Acquired	Genotype (23S)	Source collection no.	Isolated from
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Table 2. [redacted].

The nucleotide sequences of the nine different 23S genotypes along with their [redacted] accession numbers and species designation as per their respective source collection are provided in **Attachment 4** while their taxonomic relationship is diagrammed below in Figure 6. [redacted]

[redacted]

Figure 6. Cladogram based on partial, plastidic 23S rDNA sequences [redacted]

2.1.2.2 Genetic characterization [redacted]

A complete genome sequence [redacted] is available. We have carried out shotgun sequencing (Roche 454) and Illumina paired end reads as well as transcriptome analysis of the strain. The genome size of the organism is [redacted] We continue to generate additional sequence information in order to resolve ambiguous assemblies and issues around highly homologous alleles and gene families through Roche paired end as well as BAC sequencing. We have also completed work aimed at characterizing the genome size of the organism through alternative methods including contour-clamped homogeneous electric field (CHEF) gel analysis and Fulgen staining (Table 3 and figure 7) followed by FACS (Flourescent Activated Cell Sorting) analysis and these results are in excellent agreement with our sequencing work. The organism appears to be a diploid.

Organism	Genome size by sequencing (Mbp)	Genome size by Fulgen staining (Mbp)
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Table 3. Genome sizes as assessed by sequencing versus Fulgen staining.

[redacted]

Figure 7. Genome size [redacted]

[redacted]

2.1.2.3 Genetic relatedness [redacted]

There are no gross differences between the original [redacted] strain [redacted] and its classically improved and genetically modified counterparts, [redacted]. The strain modification history is shown in Figure 8.

[redacted]

Figure 8. Strain modification history. [redacted]

To demonstrate the similarity between the three strains, we assessed two parameters we had looked at earlier [redacted], namely, the migration of the strains' chromosomes based on CHEF gel analysis and their respective plastidic, 23S genotypes. Figure 9 below shows a CHEF gel analysis [redacted]. As can be seen, there are no gross differences between the migration patterns of the chromosomes between the three strains. Figure 10 shows the plastidic 23S rDNA genotype [redacted].

[redacted]

Figure 9. CHEF gel analysis of [redacted]

[redacted]

Figure 10. Plastidic 23S rDNA genotyping of three isolates each from strains [redacted]

All of the above data demonstrate that on multiple levels, there are no gross differences between [redacted]

2.2 Features of the New Microorganism

[redacted] has an active strain improvement program to [redacted] the wild-type strain[redacted] To maximize the probability of generating mutants with the desired traits, both physical and chemical mutagens are used to exploit the differences in their modes of DNA alteration. Mutants that demonstrate increased productivities such [redacted] are selected for subsequent rounds of strain improvement. Strain [redacted] was taken through a classical strain improvement program utilizing both physical and chemical mutagens, [redacted] and given the internal designation [redacted] (Figure 8 above). Morphological assessment with brightfield and fluorescence staining has shown that [redacted] is [redacted] strain (**Attachment 5**). It has also been determined that [redacted]

2.3 Morphological and Physiological Features of New Microorganism

We went on to transform the classically improved [redacted] strain with a [redacted]

[redacted]

Figure 11. [redacted]

The resulting strain was given the [redacted] and is the subject of this MCAN submission.

[redacted] has a similar morphology in terms of size and [redacted] compared to the unmodified [redacted] (**Attachment 5**) and now has the ability to [redacted]. Physiological characterizations to determine similarities and any potential differences between the original parental strain [redacted]

2.3.1 Growth rates

As shown in Table 4 there are no significant growth rate differences in these strains. [redacted]. Growth rates were determined by optical density measurements as detailed in **Attachment 6**.

Strain	Carbon Source	Growth Rate (h^{-1})
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]

Table 4. [redacted]

2.3.2 Growth temperatures

[redacted] (Figure 12). [redacted] The ability of [redacted] to grow at temperatures below [redacted] has not been examined [redacted]. However, it has been reported by [redacted]

[5] [redacted]

[redacted]

Figure 12. [redacted]

2.3.3 [redacted]

As shown in Figure 13 below, [redacted]

[redacted]

Figure 13. [redacted]

2.3.4 [redacted]

[redacted] (Figure 14). [redacted]

[redacted]

Figure 14. [redacted]

2.3.5 [redacted]

[redacted] Table 5.

[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Table 5. [redacted]

2.3.6 [redacted]

Of the three strains evaluated, [redacted] (Figure 15 below). [redacted]

[redacted]

Figure 15 [redacted]

2.4 Genetic Construction [redacted]

2.4.1 The taxonomy of the donor organism

[redacted] is a stable transformant of strain [redacted], a classically improved strain derived from [redacted] The component parts of the transgene introduced into [redacted] This is shown schematically in Figure 16 below.

[redacted]

Figure 16 [redacted]

2.4.2 Description of traits for which the microorganism was selected

[redacted]

[redacted] Additional information concerning the characteristics of the new strain can be found in Section 2.3 above.

2.4.3 Genetic modifications [redacted]

We have developed the means to transform [redacted] Transformation in [redacted] is mediated by homologous recombination and as such, integrated cassettes have excellent stability. Construction of integrative vectors requires some knowledge of host genomic sequences because the vectors integrate through homologous recombination. Integrative vectors are designed such that [redacted] is cloned and modified if necessary to contain a suitable restriction site at the approximate midpoint of the genomic DNA. Heterologous genes of interest can then be inserted into this site and the entire cassette then cloned into a suitable vector for replication in *E. coli*, such as pUC19 or pBlueScript. The resulting cassette consists of the heterologous gene now flanked on either side by what was formerly a contiguous piece of host genomic DNA, cloned into an *E. coli*-based vector.

Prior to transformation, the DNA is linearized with restriction enzymes such that the heterologous gene of interest, flanked on either side by [redacted] genomic DNA, is physically separated from the rest of the plasmid sequences necessary for maintenance in *E. coli*. Transformation of the linearized construct allows for the insertion of the heterologous gene at the precise location in the [redacted] genome defined by the flanking sequences through homologous recombination (Figure 16 above).

[redacted] Codon usage and gene optimization in [redacted]

Proper expression of transgenes requires that the codon usage of the transgene matches the specific codon bias of the organism in which it is being expressed. The codon usage in the recipient strain [redacted] was deduced from extensive analysis [redacted] cDNA sequences. All genes encoding proteins in this document have been codon optimized to ensure efficient translation of the resulting mRNA. A sequence comparison of the wild type [redacted] sequence compared to the codon optimized sequence is provided in **Attachment 11**.

2.4.5 Detailed description of the genetic construction

Figure 17 below is the complete nucleotide sequence of the transforming DNA, including its vector backbone, used to generate [redacted]

[redacted]

[redacted]

[redacted]

Figure 17. Complete coding sequence [redacted]

The [redacted]

Flanking sequences are derived from the [redacted]

[redacted]

2.4.6 Stability of Inserted Genetic Material

All modifications were integrated directly into chromosomal DNA. No loss of any inserted genetic material has been observed. The [redacted] was demonstrated to be 100% stable as follows. The primary transformant giving rise to [redacted] generated as described earlier and [redacted], was grown in liquid culture with [redacted] as the sole carbon source (non-selective conditions). After 30 generations, cells were plated to solid medium containing [redacted] as the sole carbon source. 48 individual colonies were then picked from this plate and transferred to a 96 well plate made up with medium containing [redacted] as the sole carbon source. Forty eight of forty eight clones transferred exhibited growth on the [redacted] containing plate after more than 30 generations of growth in the absence of [redacted] selection, indicating the inserted transgene is 100% stable. To further validate the stability of the transgene and its correct targeting to [redacted]

[redacted]

Figure 18. [redacted]

[redacted]

Figure 19. [redacted]

2.4.7 PCR Amplification [redacted]

[redacted]. Figure 20 shows that the primer pairs are only capable of amplifying a product in the transgenic line and it is of the correct size. The resulting PCR product was then cloned and subjected to sequencing, the results of which are shown in Figure 21. [redacted]

[redacted]

Figure 20. PCR analysis [redacted]

[redacted]

[redacted]

Figure 21. PCR analysis and [redacted]

3.0 ECOLOGICAL ASSESSMENT

3.1 Phenotype

The strain phenotype request is understood to refer to the expression of the genes of the organisms as well as the influence of environmental factors and random variation. The interaction between these factors may be represented as genotype + environment + random variation → phenotype. The strain phenotype produces [redacted]

3.2 Habitat, Geological Distribution and Source of the Recipient Microorganism

3.2.1 Description of [redacted]

Regarding the origins [redacted]

[redacted]

Figure 22. [redacted]

3.2.2 Geographical distribution [redacted]

3.2.2.1 Overview

[redacted]

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6 [redacted]

[redacted]

[redacted]

[redacted]

3.2.2.2 Aquatic environments

[redacted]

3.2.2.3 Sewage environments

[redacted]

[redacted]

3.2.2.4 Food contact

There have also been several accounts of [redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Table 6. [redacted]

Although [redacted] found in food products and thereby ingested, laboratory experiments in rodents and primates have shown that [redacted] are unharmed by the digestive process and pass through the gut into the feces with little or no reproduction or harm to the host [redacted]

3.3 Detection, Survival, and Dissemination under Relevant Environmental Conditions

3.3.1 Control technologies, inactivation protocols, and fermentation conditions

[redacted] is grown in a contained environment in a production facility, and it is highly unlikely for any viable [redacted] to be present in the final product, as [redacted] inactivate all viable cells. The organism is classified as a BSL-1 and Good Large Scale Practice (GLSP) organism (GLSP at >10 L). Control technologies at release points and inactivation protocols are in place to reduce or eliminate the dissemination of viable modified microorganisms. Please refer to Section 7.0 for further details on the air, solid waste, and wastewater inactivation treatments and containment descriptions. In addition to inactivation protocols and control technologies, as discussed in Section 7.3 [redacted] the fermentation process engineering controls and protocols, restrict the ability to reproduce, disseminate, and spread outside the contained vessel. [redacted]

[redacted]

Figure 23. [redacted]

3.3.2 Description of method for detecting the microorganism in the environment

Section 2.4.7 of the MCAN provides details concerning a method for detecting the microorganism in the environment utilizing PCR analysis.

3.4 Anticipated Biological Interactions with Target Organisms and Other Organisms

[redacted]Host range: The range of host species or cell types which the modified production strain is able to infect or parasitize is expected to be no different than that for the recipient strain that is typically used in chemical production today[redacted]

- Target organism: The use of the production strain is [redacted]. The microorganism is not designed to act upon a particular organism during the production process or otherwise.
- Competitors: During fermentation, competing microorganisms include normal airborne microorganisms such as [redacted] that are considered to be contaminants. If left unchecked, they will compete with the production organism for nutrients and resources, which will cause stress to the modified microorganism, which will reduce yields [redacted] Therefore, aseptic techniques are employed during the whole process from seed propagation to fermentation.
- Prey: The host and modified microorganism is not designed to prey upon living organisms as a food source.
- Hosts: The use of the production strain is for [redacted] The microorganism is not designed to be a host or to infect or feed upon another living organism.
- Symbionts: The production strain is not designed to be an organism in a symbiotic relationship. It is not designed to serve as a host in which the presence of a smaller symbiont beneficiary would be present.
- Parasites: No significant interactions with parasites were discussed in a review of publically available scientific literature.
- Pathogens: No significant interactions with pathogens were discussed in publically available scientific literature. The production strain or host strains are not designed to enhance any pathogens such as *Escherichia coli* O157, *Clostridium botulinum*, etc.

3.4.1 Effects on plants

There are no published studies on the effects of [redacted] the recipient microorganism of this MCAN, on plants. However, there are studies available that describe the effects of [redacted] that we have used as surrogate data to evaluate plant pathogenicity. [redacted] Published evidence indicates that several different species of plants serve as a natural habitat for [redacted] with no reports of adverse effects. Therefore, species in the genus [redacted] are unlikely to be a plant pathogen.

3.4.2 Effects on animals

Although the presence of [redacted]

[redacted]

As noted, several animal studies demonstrate [redacted] In addition, we have summarized other animal studies in the human health hazard assessment section 4.0.

3.4.3 Potential for gene transfer

We were unable to locate any published articles discussing the potential for gene transfer between [redacted] and other organisms. Furthermore, we were unable to locate any published articles on laboratory studies related to gene transfer between [redacted] and other organisms. [redacted]

Therefore, we have based our analysis on potential for gene transfer between microorganisms in general. Microorganisms in the wild are constantly being exposed to DNA with an estimated 0.2-19 µg DNA/L in marine aquatic environments, 0.5-25.6 µg DNA/L for freshwater environments, 1 mg/DNA/g of freshwater sediment, and 80-85 µg DNA/g of soil [redacted] DNA, although abundant in the environment, is not stable due to chemicals and nucleases present in the environment, and DNA may also bind to other solids. The half-life of DNA in wastewater is 1-13.8 minutes, 4.2-5.5 hours in freshwater, 3.4-83 hours in marine waters, and 9.1-235 hours in sediments and soils [redacted] In addition, the recipient microorganism needs to be in a competent state and under ideal environment conditions (i.e., temperature shifts, specific cautions, or electrical pulses) in order for gene uptake to occur, and most prokaryotes, which are typically the group of microorganisms to participate in genetic transfer from the environment, are not competent in the wild [redacted] Therefore, it is not surprising to note that despite the detection of transgene from genetically modified plants in the soil, no gene transfer has been documented to occur to any of the soil microbial population [redacted] Uptake of genes from the modified [redacted] requires the host (recipient) microorganism to have proper intracellular DNA stabilization mechanisms, accurate expression of the gene, and appropriate posttranslational modification mechanism [redacted] Furthermore, the intergeneric gene inserted into the modified [redacted]

3.4.4 Interactions in the environment – e.g., biogeochemical cycling, trophic level, competitiveness, non-targets, etc.

3.4.4.1 Recipient microorganism

There is very little information in the public literature that specifically addresses the ecological role of [redacted]. role in the environment is as [redacted]. As [redacted]

3.4.4.2 MCAN microorganism

The modified strain expresses a [redacted]

Based on Table 4, the growth rate of [redacted] Therefore, growth characterizations of the modified strain show that it is unlikely to grow faster than the unmodified [redacted] strain.

[redacted] (Figure 15).

4.0 POTENTIAL HUMAN HEALTH HAZARD ASSESSMENT

[redacted] has conducted a thorough assessment on the safety of both the host organism [redacted] and the genetic elements used to generate [redacted]

[redacted] are classified as Biosafety Level 1 (BSL-1) and have no known hazard to laboratory personnel. These strains are non-pathogenic, harmless organisms. Standard microbiological practices consistent with BSL-1 organisms for production volumes at or below 10 L should be followed when working with this strain (Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health, February 2007). For volumes greater than 10 L, GLSP level treatment is appropriate for this organism. Both the facilities in [redacted] have levels of release controls beyond the requirements of GLSP such as [redacted]

Based on available scientific and patent literature, [redacted] are safe. The genetic elements used to generate [redacted] are well characterized, come from well-known and studied microorganisms, and have no known risks.

In addition, since [redacted]. [redacted] has performed a significant number of stirred tank fermentations and shake flasks experiments using [redacted] an Institutional Biosafety Committee (IBC) comprised of technically qualified individuals, which has not observed or reported any adverse health effects during the production of [redacted]

[redacted] assessed the safety of strains [redacted] and determined that they are non-pathogenic organisms that present no known hazard to laboratory personnel. The [redacted] IBC determined that [redacted] was suitable to be run under GLSP conditions (**Attachment 18**). Risk Assessments of Transgenic Strains [redacted] also describe the safety assessment of newer derivative strains. Strains [redacted] Copies of these documents are provided in **Attachment 19**.

Other [redacted] strains, including [redacted] were cultivated at a laboratory scale [redacted] with no known or observed adverse health effects. In addition, [redacted] fermentations [redacted] with no known or observed adverse health effects. [redacted] has performed [redacted] at laboratory scale [redacted] with no known or observed adverse human health effects.

Altogether, both at the pilot scale and laboratory scale, [redacted]

[redacted]

[redacted] is classified as a BSL-1 organism by ATCC and is not listed as pathogenic by UTEX.

[redacted] has concluded that it does not have any information concerning any significant adverse reactions of persons exposed to [redacted] either through direct experience or from publicly available sources of information. The supplier of the host strain used to generate [redacted] has not provided [redacted] with information concerning any health risk believed to be associated with the host strain despite its practice of doing so if it has such information.

Extensive small–scale and large-scale cultivation of [redacted] has not generated any negative health or environmental effect data. [redacted] is not aware of any information on health effects that accompany any EPA rule or order issued under TSCA section 4, 5, or 6 that applies to [redacted] Based on this assessment, [redacted] concluded that cultivation of [redacted] has no known health risks.

4.1 History of Safe Use

4.1.1 Recipient microorganism

This species, [redacted] has not yielded any known or observed negative effects on the safety or health of personnel.

[redacted]

[redacted]

[redacted]

4.1.3 Donor organism

[redacted]

4.2 Potential for human pathogenicity

The recipient strain has been classified as a BSL-1 organism by ATCC [redacted] which means that it is not known to cause disease in healthy adult humans.[8] Our review of primary research articles below concurs with the BSL-1 assessment that [redacted] is a rare and opportunistic pathogen and not a vector for pathogens.

[redacted]

4.2.3 Pathogenicity/toxicity

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

4.2.4 Submission microorganism – effect of genetic modifications on microorganism pathogenicity potential, behavior, effects of products

The modified [redacted] compared to the unmodified recipient [redacted] strain [redacted] This is not anticipated to increase the pathogenicity potential of the modified [redacted] The growth rate of the modified [redacted] strain is the same or less than both the original parental strain [redacted]

4.3 Allergenicity

There are no published reports of any members of the [redacted] as a potential allergen. However, as [redacted] is taxonomically related to [redacted] and there are published studies on the allergenic potential of [redacted] we have based our assessment of allergenicity on [redacted] as surrogate data for consideration. In a study that assayed for IgE antibodies specific for [redacted] skin prick tests and a conjunctival provocation test concluded a higher incidence of a positive reaction to [redacted] could be found in children who were already sensitive to mold or other allergens [redacted] However, the conclusion in this paper was that [redacted] was a “weak allergen” that may be clinically important to populations sensitive to other allergens.

4.4 Virulence

The ability for an organism to be pathogenic is promoted by the presence of virulence factors, such as proteases, lipases, and toxins. Because the gene modification to the organism was not shown through a literature search to be toxic or yield different toxicological results from the parental strain, surrogate information on the recipient strain is offered for the purpose of evaluating the anticipated behavior of the production strain.

4.5 Immunologic Reactions

The inserted genetic elements in this case do not appear to possess any intrinsic hazard potential given the history of safe use of the enzyme, and the gene modification to the organism was not shown through a literature search to be toxic or yield different immunological or toxicological results from the parental strain.

4.6 Antibiotic Resistance (ABR)

The final production strain does not contain any ABR genes, and therefore the modified microorganism is as susceptible to antibiotics and to anti-fungals as the unmodified microorganism[redacted]

A PubMed and a Google Scholar search did not produce any articles related to resistance to antibiotics when we conducted a search using the terms [redacted] in conjunction with metal, pesticide, or herbicide. A null result was expected since [redacted] is not known to have any effect upon the survivability of the [redacted]

Since the inserted genetic elements in this case do not appear to possess any intrinsic ability to confer antibiotic resistance, data are being provided for the species in general based on the rationale that the gene modification to the organism has not been shown, through a literature search, to impact antibiotic resistance *per se*.

Based on the absence of demonstrated adverse effects for the parental strain and for the inserted intergeneric sequence, it is reasonable to conclude that the modified strain is not expected to be any different from other well-known [redacted] strains commonly found in nature.

4.7 Action as a Vector for Pathogens

A PubMed and Google Scholar search using the terms [redacted] and pathogen* and "vector" does not return any articles, demonstrating that the presence of the [redacted] alone is unlikely to permit the production strain to act as a vector of pathogens. A null result was expected since [redacted] There are no studies that the submitter could locate that would indicate the donor strain itself, [redacted] acts as a vector for pathogens. Because the gene modification to the organism was not shown through a literature search to yield different results from the parental strain, surrogate information on the recipient strain is offered for the purpose of evaluating the anticipated behavior of the production strain. The production strain is not expected to act as a vector of any pathogen such as *Escherichia coli* or *Clostridium botulinum.*

5.0 MANUFACTURING PROCESS, USE, AND DISPOSAL OF THE STRAIN

5.1 Maximum Amount to be Produced in Year One and Year Three (by volume) and Estimation of Viability (cells per unit volume or cfu)

The modified strain will be used to produce [redacted] in the United States. The expected volumes are as shown below in Table 7.

YEAR	# of FERMENTORS	MAX # BATCHES	UTIL RATE	# of BATCH/ Year	CFU per mL BROTH	CFU per BATCH	**CFU per YEAR (CFU)**	Total fermentor VOL per BATCH (L)	Average fermentation time/batch hours
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Table 7. Estimated production volumes for a three-year period. Please refer to Attachment 12 for the laboratory protocol.

[redacted]

Utilization rate (“UTIL RATE”) refers to the percentage of time during that year that the Submitter anticipates that the manufacturing facilities will be operational. To capture the most accurate total viable cell counts, [redacted]

5.2 Manufacturing Process Description

5.2.1 Operational description

The [redacted] facility is a fermentation and processing plant for [redacted] chemicals. The [redacted] are grown in closed fermentors, and the entire site provides containment for the use of such microorganisms through the use of berms, trenches and sumps which channel all liquid to tanks where the cells can be inactivated by heat or chemical means.

Microorganisms are handled at the appropriate level of biosafety (BSL-1, GLSP) as designated by CDC/NIH guidelines. Handling of effluent streams, solid, liquid, and gaseous, are all designed to provide the appropriate reduction and control of the microorganism to minimize any off site releases.

The identity of the site at which the operation will occur is [redacted], for the fermentation and subsequent post-fermentation processing [redacted]

5.2.2 Transportation

Master cultures are stored in [redacted] Maintenance and storage of the master culture stock will be at the Submitter's laboratory under the supervision of a qualified laboratory microbiologist. Cell propagation, growth, [redacted]

To transport the master culture stock [redacted] vials will be placed in a double zip-lock bag within a Styrofoam container. The container will be filled with dry ice and transported *via* commercial shipper *(e.g.* Federal Express or equivalent) in accordance with Department of Transportation Hazardous Materials requirements. The container will include a material safety data sheet (MSDS) that describes the nature of the contents (**Attachment 29**). Instructions given on the MSDS describe containment methods for an accidental release and disposal of the material. Upon receiving a master stock culture, a staff microbiology technician at the facility, using standard laboratory procedures, will propagate the culture and prepare a working stock culture. [redacted]

5.2.3 Cell propagation

[redacted]

Figure 24. Cell propagation diagram.

A diagram for cell propagation and fermentation is shown in Figure 24 above. The master culture vial contents will be transferred into [redacted] flask, containing growth media, using a pipette and following aseptic techniques. This initial transfer will take place inside a bio hood

[redacted]

After the desired cell density is obtained, the contents of the [redacted] will be transferred to a [redacted] following standard aseptic techniques. This transfer will take place inside a bio hood.

[redacted]

After the desired cell density is obtained, the contents [redacted] will be transferred into a [redacted] [redacted]

[redacted] The connection is then sterilized by steam after the flask hose is attached. [redacted] will be cleaned through chemical inactivation methods such as bleach, and autoclaved for the next use. Bleach has been shown to be effective in inactivation [redacted]

[redacted]

[redacted]

[redacted]

5.2.4 Fermentation

[redacted]

[redacted]

[redacted]

[redacted]

All process water from cleaning and related activities will be collected and sent to an on-site waste treatment area, where cells will be treated using heat, chemicals, or other suitable means to ensure inactivation.

All samples taken during the fermentation stages will be taken from a sample port and collected in sterile containers. The containers will be taken directly to the facility's laboratory for analysis. Broth will be purged from the ports prior to sampling and sent to an on-site waste treatment area, which will inactivate any residual cells through heat or chemical treatments.

[redacted]

Figure 25[redacted]

5.2.4 [redacted]

[redacted]

[redacted]

[redacted]

[redacted]. The remaining air will be released into the atmosphere by dryer exhaust fans. [redacted]

5.2.5 [redacted]

[redacted]

5.2.6 [redacted]

[redacted]

5.2.7 Waste disposal and sterilization procedures

[redacted] will be sterilized in the autoclave for re-use. With respect to sterilization of the transfer hose or lines connecting the fermentation tanks, these will also be steam-sterilized.

After a tank has been emptied, the tank must be rinsed with water at the conclusion of the run. The rinse water will then be sent to an on-site waste treatment area, where cells will be treated using heat, chemicals, or other suitable means to ensure inactivation. After rinsing the tank, it will be heat-sterilized with steam [redacted] The tank sterilization will be completed before any subsequent runs. [redacted] with liquids [redacted] being sent to an on-site waste treatment area.

[redacted] the transfer hoses will all be flushed with water after use. The rinse water will then be sent to an on-site waste treatment area.

[redacted]

5.3 Product Description

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Table 8: [redacted]

[redacted]

Figure 26 [redacted]

6.0 WORKER EXPOSURE INFORMATION

The manufacturing site will be under the control of the Submitter. The production strain is currently intended for use in the [redacted] which will meet the containment criteria to qualify for the full Tier 1 exemption. Future production may expand to include other facilities that will also meet the containment criteria to qualify for the full Tier 1 exemption. Workers will be grouped into shifts consisting of [redacted] people [redacted] Lab Technician, [redacted] Production Technicians, [redacted] Operator/Mechanic, and [redacted] Shift Supervisor (Table 9 below). Shift teams provide coverage 24 hours/day, 7 days/week. Additional personnel may be on shift, depending on workload.

Lab Technicians are responsible for the storage of working stock cultures, preparation of inoculum (flask growth stages), and analyses of fermentation samples. Production Technicians are responsible for operation of all manufacturing steps, [redacted] Maintenance Operator/Mechanics are primarily responsible for addressing maintenance issues which arise during operations, coordinating with Plant Site Maintenance Department for assistance as needed, and assisting the production operators with plant operations. Engineers/Tech Managers and Roving Technicians provide technical resources and supervision of all activities that occur on their shift.

Position	Activity	PPE/Eng Control	Max # workers involved in each activity	Max duration Hours/day Days/year
Production Technicians "Roving Techs"	Sampling, Cleaning fermentors Inoculating Process support	Gloves, safety glasses, lab coats	[redacted] [redacted] [redacted]	[redacted] Actual exposure limited by engineering controls, PPE. Handling of cultures while sampling less than 1 hour/day
Lab Technicians	Culture transfer, shake flask inoculation	Gloves, safety glasses, lab coats	[redacted]	8 hour shifts 250 days/year Handling of cultures while sampling less than 1 hour/day
Engineer/Tech Manager	Process oversight	Gloves, safety glasses, lab coats	[redacted]	8 hour shifts 250 days/year
Maintenance Operator Mechanics	Repair of fermentation equipment: valves, pumps, etc.	Gloves, safety glasses	[redacted]	8 hour shifts 250 days/year

Table 9: Worker exposure.

Exposure will be negligible, as the fermentation process is conducted in a totally closed system from which samples are drawn approximately once every 2-4 hours for in-process analysis. This sampling will only take about 1 minute. The Lab Technician is in charge of in-process sampling, but Production Technicians may also take samples.

In addition, facility operations are tailored to minimize exposure of personnel to viable microorganisms and to restrict releases of viable microorganisms into the environment. Employees will all be trained in aseptic techniques and in the potential health risks of microorganisms. Further, access to the fermentation lab and production areas – indeed, to the entire plant site – is strictly controlled and limited to employees and authorized visitors. Microorganisms are only handled in a manner intended to safeguard personnel health, through standard PPE and procedures.

7.0 POTENTIAL RELEASE INTO THE ENVIRONMENT

Cells from air exhaust, liquid, and solid waste are captured through extensive control technologies and inactivation protocols as described in Table 10 below.

Release	New substance released CFU/Day to Environment/ Control Technology	Media of Release	Control Technology	Efficiency
Process Wastewater	The site will inactivate viable cells, collect and treat wastewater, [redacted] **Minimal/no release to the environment.**	Waste rinse water from cleaning of fermentors, transfer lines.	Heat or chemical inactivation in treatment tanks.	6 log reduction or greater, based on samples taken after treatment.

Solid waste from lab	Daily plates, and other solid waste. **no release to the environment**. All solid waste from labs autoclaved. [redacted]	Agar plates and other solid lab waste contaminated with the culture.	Autoclaved to ensure sterility.	6 log reduction or greater based on heat inactivation tests of biomass after drying.
Exhaust from fermentors	**Minimal/no release to the environment.** [redacted] liquid waste, which is treated before release	Air exhaust from fermentors [redacted]	[redacted] capture and condensate out potential aerosols into wastewater, where they will be treated with heat or chemicals before release.	[redacted]
Lab sink waste	[redacted]	Sample bottles	All samples	6 log

	day 5 days /week from in process sampling. **no release to the environment**. All waste from labs autoclaved or chemical treated. [redacted]	washed in lab sink going to drains that do not connect to treatment tanks.	are autoclaved or treated with chemicals to ensure inactivation before being washed in sinks.	reduction or greater based on chemical and/or heat inactivation data.

Table 10. Releases and control technology. *Cells from air exhaust, liquid, and solid waste are captured through extensive control technologies and inactivation protocols.*

7.1 Air Release

7.1.1 Fermentation

Air exhaust from the [redacted] vapor droplets (see Figure 24 Cell Propagation Diagram). Steps have been taken to minimize this exposure. [redacted] a vent allows air and smaller vapor particles to escape, while being designed to restrict liquid releases via the exhaust air from the structure. The liquid releases are captured in a liquid stream, which is sent to the on-site wastewater treatment plant. With these controls in place, expected emissions [redacted] are estimated to be insignificant.

7.1.2 [redacted]

[redacted]

[redacted]

7.2 Wastewater Release

[redacted] will produce lab scale wastewater from washing vials and flasks that come into contact with [redacted]. Lab ware containing viable cells is chemically treated or autoclaved before washing to ensure inactivation.

Process wastewater is generated in the fermentors and tanks [redacted] and collecting liquid vapor from [redacted] The wastewater produced will be handled on-site at a wastewater treatment plant (WWTP) in accordance with a [redacted] wastewater discharge permit from the [redacted] The facility will discharge according to the permit requirements [redacted]

Treatment steps included in the [redacted] WWTP serve to restrict the release of viable [redacted] into the environment. Critical elements of the wastewater treatment system that support this determination include:

- Heat or chemical treatment to inactivate organisms;
- Neutralization of the wastewater to pH level 6.2-9.0;
- Removal of floatable and settable solids through flocculation and secondary clarifiers. A portion of this sludge is then returned to the WWTP's aeration basins; another portion is sent back to the wet well for reprocessing; and the remaining 8-15% is collected, dewatered and transported off-site for incineration; and
- Discharge of clarified effluent after combining with storm water and non-contact coolant water in accordance with the WWTP's permit conditions.

Because of these treatment steps, and our knowledge that the modified [redacted] at issue is neither [redacted] will be restricted in effluent discharge.

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10 [redacted]

[redacted]

7.2.1 Failed fermentation broth

Viable [redacted] will exist in the fermentation broth. Thus, in the event that the entire contents of a fermentation vessel must be discarded due to a "failed" run, the contents will be inactivated by heating to greater [redacted] prior to discharging the broth to the WWTP. The heat step results in at least a 6 log reduction in total viable cells as our data demonstrates greater than 7 log reduction when cells are treated at a lower temperature [redacted] (**Attachment 31**).

7.3 Solid Waste

Solid waste from lab activities will be inactivated with chemicals or heat (autoclaving) to ensure viable cells are not present in lab waste. Please refer to Section 5.2.7 entitled "Waste Disposal and Sterilization Procedures" for details regarding the disposal of inactivated solid waste.

7.3.1 [redacted] drying

Viable [redacted] will not survive the on-site drying process. The final fermentation broth containing the viable [redacted] will be exposed to temperatures ranging from [redacted], conditions that will inactivate the cells (see **Attachment 32**). Similarly, final fermentation broth to be sent off-site for drying will also go through a heat treatment step: the fermentation broth will be heated to greater [redacted] minutes, which are conditions that will inactivate the cells (**Attachment 31**). These protocols are designed so that viable [redacted] will not survive the drying process, and therefore viable [redacted] will not be present in the [redacted] is then sent to landfill in accordance to local regulations and authorities.

7.4 Dissemination and Spread of Viable [redacted]

The fermentation process engineering controls restrict the functional ability of [redacted] to reproduce, disseminate, and spread. Specifically, the conditions in the fermentor are designed to hold the organism at a physiological state at which they [redacted] The entire fermentation takes place an enclosed vessel, with several stages of containment to prevent offsite releases. The threat of viable [redacted] spreading into the environment during the fermentation process is negligible.

As discussed in Section 7 above, cells from air exhaust, liquid, and solid waste are captured through extensive control technologies and inactivation protocols.

8.0 PROCEDURES FOR DISPOSAL OF ARTICLES, WASTE, CLOTHING, AND EQUIPMENT

All laboratory waste known to have been in contact with active biological materials are disposed of in designated biohazard waste receptacles. Biohazard waste is collected and killed using an autoclave heated to >121° C. Once deactivated this waste is disposed of via a private garbage handler with normal waste streams.

Lab clothing is collected and cleaned by an industrial service. Clothing also can be cleaned using a normal household detergent.

All other equipment, including bench tops are disinfected using commercial antimicrobial cleaner or equivalent after coming into contact with a modified microorganism. When acceptable, equipment will be placed into an autoclave and heated to >121° C in order to decontaminate prior to re-use.

9.0 SPILLS AND EMERGENCY PREPAREDNESS MEASURES

Good Microbiological Lab Practices and Good Large Scale Practices for BSL-1 and GLSP will be employed. Personnel routinely utilize the following practices in their normal operation to minimize exposure of personnel to viable microorganisms, and to restrict release of viable microorganisms to the environment. All of these practices must be utilized while conducting processes involving transgenic strains:

1. Employees are trained in aseptic techniques and in the potential health risks of microorganisms utilized so that potential biohazards can be understood and appreciated;

2. Access to fermentation lab and production areas, and the entire Plant site, is strictly controlled and limited to employees and authorized visitors;

3. Strains are handled in facilities intended to safeguard personnel health during handling;

4. Work surfaces where strains are handled are cleaned and decontaminated at least once per day with one or more of isopropyl alcohol, ethanol, Vesphene IISE, LpH se, or equivalent;

5. Contaminated wastes are either disposed via the lab sink which drains to the holding tanks (*e.g.*, broth samples after testing has been completed), or autoclaved or bleached prior to disposal (*e.g.*, used pipettes, used sample cups, used agar plates, empty cryovials);

6. Mechanical pipetting devices are used; mouth pipetting is prohibited;

7. Eating, drinking, smoking, and applying cosmetics are not permitted in the work areas. Food may only be stored in cabinets or refrigerators designated and used for this purpose only;

8. All persons wash their hands after they handle materials involving live strains;

9. In the interest of good personal hygiene, facilities (*e.g.*, hand washing sink, shower and change room) and protective clothing (*e.g.*, uniforms, lab coats, and safety glasses) are provided;

10. An insect and rodent control program is in effect;

11. The lab has windows that cannot be opened; the lab is air-conditioned;

12. Sample collection, transfer of culture fluids within/between systems, and processing of culture fluids are conducted in a manner that minimizes release of aerosols and maintains employee exposure to viable cells at a level that does not adversely affect the health and safety of employees;

13. A closed system which contains, or has contained, viable cells (e.g., a fermentation vessel) shall not be opened for maintenance until it has been thoroughly washed and sterilized by heat, chemicals or equivalent;

14. The site has institutional codes of practice that have been formulated and implemented among all employees to assure adequate control of health and safety matters; these include an industrial hygiene program, safety program and procedures, training, and periodic refresher training; and

15. The site has an emergency response plan, which includes provisions for handling spills.

10.0 PROCEDURES FOR TERMINATING THE ORGANISM

All fermentation broth is intended for [redacted], but on occasion, a fermentation batch may perform poorly or become accidentally contaminated with a competing organism. Cross-contamination incidents will be unlikely due to SOPs, including cleaning of the fermentors before and after use. However, in the event of cross-contamination or for other contamination reasons, termination may be desired. In such a case, the fermentation broth and all other liquid in the process would be processed with heat inactivation at temperature and time treatments or chemical treatment known to inactive the modified microorganisms. The fermentation equipment would undergo CIP rinses, heat sterilization and all wastewater and CIP liquids are treated to inactive the modified microorganism. Inactivation can be confirmed by samples plated onto growth media to show no viable [redacted] are present in the treated wastewater.

11.0 HEALTH AND SAFETY DATA

As required by 40 C.F.R. §725.160, all testing regarding the health and environmental effects conducted on the microorganism known to the Submitter have been provided with this MCAN. Copies of the studies and references in the Submitter's possession and control have also been provided with this MCAN.

12.0 PROCESS DECRIPTION OF SITES NOT CONTROLLED BY THE SUBMITTER

The production strain is currently intended for use [redacted] which will meet the containment criteria to qualify for the full Tier 1 exemption. However, future production may expand to include other facilities that will also meet the containment criteria to qualify for the full Tier 1 exemption.

[redacted]